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L12: Entry 1 of 1 File: USPT Dec 17, 2002

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TITLE: Flow cytometric, whole blood dendritic cell immune function assay

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INVENTOR-INFORMATION:

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CLAIMS:

What is claimed is:

1. A method of measuring dendritic cell function wherein each step in said method is sequentially performed on one whole blood sample, said method comprising: (a) contacting a sample of whole blood with a dendritic cell activator and a secretion inhibitor; (b) contacting said sample with a plurality of dendritic cell distinguishing antibodies and at least one dendritic cell subsetting antibody; (c) permeabilizing nucleated cells in said sample with a permeabilizing agent; (d) contacting said sample with at least one cytokine-specific antibody, said cytokine-specific antibody binding to cytokine inside the cell resulting in intracellular binding; and then (e) flow cytometrically measuring said intracellular binding of said at least one cytokine-specific antibody by dendritic cells that concurrently bind said dendritic cell subsetting antibody, the level of intracellular binding providing a measure of dendritic cell function.

2. The method of claim 1, further comprising the step, after step (b) and before step (c), of lysing erythrocytes in said sample with a lysing agent.

3. The method of either claim 1 or 2, wherein said secretion inhibitor is selected from the group consisting of brefeldin A and monensin.

4. The method of claim 3, wherein said secretion inhibitor is brefeldin A.

5. The method of either claim 1 or claim 2, wherein said dendritic cell activator is selected from the group consisting of lipopolysaccharide (LPS), phorbol 12-myristate 13 acetate plus ionomycin (PMA+I) and a CD40-crosslinker.

6. The method of claim 5, wherein said dendritic cell activator is LPS.

7. The method of claim 5, wherein said dendritic cell activator is PMA+I.

8. The method of claim 5, wherein said dendritic cell activator is a CD40

crosslinker.

9. The method of claim 1 or claim 2, wherein at least one of said plurality of dendritic cell distinguishing antibodies is specific for a non-dendritic cell lineage.

10. The method of claim 9, wherein each of said nondendritic cell lineage-specific antibodies is specific for an antigen selected from the group consisting of CD3, CD14, CD16, CD19, CD20, and CD56.

11. The method of claim 10, wherein said plurality of dendritic cell distinguishing antibodies are collectively specific for CD3, CD14, CD16, CD19, CD20 and CD56.

12. The method of claim 11, wherein all of said nondendritic cell lineage-specific antibodies are conjugated to an identical fluorophore.

13. The method of claim 12, wherein said fluorophore is fluorescein isothiocyanate (FITC).

14. The method of claim 1 or claim 2, wherein said plurality of dendritic cell-distinguishing antibodies includes an antibody specific for HLA-DR.

15. The method of claim 1 or claim 2, wherein said plurality of dendritic cell-distinguishing antibodies includes an antibody specific for CD4.

16. The method of claim 1 or claim 2, wherein said dendritic cell subsetting antibody is selected from the group consisting of antibodies specific for CD11c and antibodies specific for CD123.

17. The method of claim 16, wherein said dendritic cell subsetting antibody is specific for CD11c.

18. The method of claim 16, wherein said dendritic cell subsetting antibody is specific for CD123.

19. The method of claim 1 or claim 2, wherein said cytokine-specific antibody is specific for an interleukin.

20. The method of claim 1 or claim 2, wherein said cytokine-specific antibody is specific for an interferon.

21. The method of claim 1 or claim 2, wherein said cytokine-specific antibody is specific for a cytokine selected from the group consisting of TNF.alpha., IL-1.beta., IL-6, IL-1RA, IL-8, IL-12 and IL-1.alpha..

22. A method of measuring dendritic cell function, comprising: (a) contacting a sample of whole blood with a dendritic cell activator; (b) contacting said sample with a plurality of dendritic cell distinguishing antibodies, at least one dendritic cell subsetting antibody, and at least one antibody specific for a cytokine; and then (c) flow cytometrically measuring the binding of said at least one cytokine specific antibody that concurrently bind said dendritic cell subsetting antibody,

the level of binding of the antibody specific for the cytokine providing a measure of dendritic cell function.

23. The method of claim 22, wherein said cytokine specific antibody is specific for antigen selected from the group consisting of CD25, CD40, CD80, CD86, HLA-DR and HLA-DQ.

24. A method of distinguishing dendritic cell subsets wherein each step in said

method is sequentially performed on one whole blood sample, said method comprising: (a) contacting a sample of whole blood with a dendritic cell activator and a secretion inhibitor; (b) contacting said sample with a plurality of dendritic cell distinguishing antibodies; (c) permeabilizing nucleated cells in said sample with a permeabilizing agent; (d) contacting said sample with at least one cytokine-specific antibody, said cytokine-specific antibody binding to cytokine inside the cell resulting in intracellular binding; and then (e) flow cytometrically measuring said intracellular binding of said at least one cytokine-specific antibody by dendritic cells,

wherein said dendritic cell subsets are distinguished by differences in the amount of said intracellular binding.